

CLAIMS

1 (amended). A stable lyophilized formulation obtainable by
lyophilization of an oil-in-water emulsion comprising a bacterial
5 component, an oil, a surfactant, and a stabilizer, as well as an amino
acid or urea, which emulsion is characterized in that:

(a) the bacterial component is encapsulated in an oil droplet;
(b) the oil droplets are dispersed with a single peak of particle
diameter distribution in the aqueous solution; and

10 (c) the particle diameter distribution of the oil droplets and
turbidity in the aqueous solution are not largely changed before and
after lyophilization.

2. The stable lyophilized formulation of claim 1, which is formed
into an aqueous solution, of which the turbidity is changed by 50% or
15 less compared to that of an aqueous solution before lyophilization.

3. The stable lyophilized formulation of claim 1 or 2, wherein
the bacterial component is a BCG-CWS, and the oil is squalane.

4. (deleted)

5. (amended) The stable lyophilized formulation of ^{claim 1} ~~any one of~~
20 ~~claims 1 to 3~~, wherein the amino acid is glycine.

6. (amended) A process for preparation of a stable lyophilized
formulation, which comprises lyophilizing an oil-in-water emulsion
comprising a bacterial component, an oil, a surfactant, and a stabilizer
as well as an amino acid or urea, which emulsion is characterized in
25 that:

(a) the bacterial component is encapsulated in an oil droplet;

(b) the oil droplets are dispersed with a single peak of particle diameter distribution in the aqueous solution; and

(c) the particle diameter distribution of the oil droplets and turbidity in the aqueous solution are not largely changed before and after lyophilization.

7. The process for preparation of a stable lyophilized formulation of claim 6, which formulation is formed into an aqueous solution, of which the turbidity is changed by 50% or less compared to that of an aqueous solution before lyophilization.

8. The process for preparation of a stable lyophilized formulation of claim 6 or 7, wherein the bacterial component is a BCG-CWS, and the oil is squalane.

9. (deleted)

10. (amended) The process for preparation of a stable lyophilized formulation of ^{claim 6} ~~any one of claims 6 to 8~~, wherein the amino acid is glycine.

11. (amended) A process for preparation of an oil-in-water emulsion wherein the emulsion is negative for agglutination reaction with lectin, and a bacterial component that is selected from a BCG-CWS and *Nocardia rubra*-CWS is encapsulated in an oil, which comprises the following steps:

(a) stirring a mixture of a bacterial component that is selected from a BCG-CWS and *Nocardia rubra*-CWS, an oil, and a dispersion-aiding solvent to disperse the bacterial component in the mixture;

(b) evaporating off the dispersion-aiding solvent to form an oil wherein the bacterial component is homogeneously dispersed, or an oil

droplet wherein the bacterial component is encapsulated in the oil; and then,

(c) adding an aqueous solution containing a surfactant thereto, and emulsifying the mixture.

5 12. (deleted)

13. The process for preparation of an oil-in-water emulsion of claim 11, wherein the bacterial component is a BCG-CWS, and the oil is squalane.

10 14. (amended) The process for preparation of an oil-in-water emulsion of claim 11 or 13, wherein the dispersion-aiding solvent is ethanol or toluene.

15 15. (amended) The process for preparation of an oil-in-water emulsion of ^{claim 11} ~~any one of claims 11, 13 and 14~~, wherein the oil droplet is dispersed in a manner that the diameter of the particle is about 100 μm or less.

16. A process for preparation of an oil-in-water emulsion, which comprises the following steps:

20 (a) stirring a mixture of a bacterial component, an oil, and a dispersion-aiding solvent to disperse the bacterial component in the mixture;

(b) evaporating off the dispersion-aiding solvent; and then;

(c) performing the following two-step emulsification process which comprises adding an aqueous solution containing a surfactant:

25 i) adding an aqueous solution containing a surfactant at a low concentration to the evaporated material, and stirring gently the mixture, thereby performing rough emulsification; and

ii) optionally, adjusting the concentration of the surfactant in the roughly emulsified solution, and stirring vigorously the mixture; thereby performing adequate emulsification in order to provide a desired particle diameter distribution.

5 17. The process for preparation of an oil-in-water emulsion of claim 16, wherein an amount of the surfactant in the aqueous solution containing the surfactant at a low concentration used in rough emulsification of the two-step emulsification process is 10% or less of the oil.

10 18. The process for preparation of an oil-in-water emulsion of claim 16 or 17, wherein the surfactant is Polysorbate 80 (Tween80).

15 19. The process for preparation of an oil-in-water emulsion of claim 16 ~~any one of claims 16 to 18~~, wherein the bacterial component is a BCG-CWS or a CWS of *Nocardia rubra*.

20 20. The process for preparation of an oil-in-water emulsion of claim 16 ~~any one of claims 16 to 19~~, wherein the bacterial component is a BCG-CWS, and the oil is squalane.

25 21. (added) An oil-in-water emulsion wherein the emulsion is negative for agglutination reaction with lectin, and a bacterial component that is selected from a BCG-CWS and *Nocardia rubra*-CWS is encapsulated in an oil, which emulsion is obtainable by the following steps:

(a) stirring a mixture of a bacterial component that is selected from a BCG-CWS and *Nocardia rubra*-CWS, an oil, and a dispersion-aiding solvent to disperse the bacterial component in the mixture;

(b) evaporating off the dispersion-aiding solvent to form an oil

wherein the bacterial component is homogeneously dispersed, or an oil droplet wherein the bacterial component is encapsulated in the oil; and then,

- 5 (c) adding an aqueous solution containing a surfactant thereto, and emulsifying the mixture.

22. (added) The oil-in-water emulsion of claim 21, wherein the dispersion-aiding solvent is ethanol or toluene.

23. (added) The oil-in-water emulsion of claim 21 or 22, wherein the bacterial component is a BCG-CWS, and the oil is squalane.

10 24. (added) An oil or an oil droplet wherein a bacterial component that is selected from a BCG-CWS and *Nocardia rubra*-CWS having an particle diameter of about 100 μm or less is homogeneously dispersed.

15 25. (added) The oil droplet of claim 24, havwherein the particle diameter is about 25 μm .

09742750-0524260